SURVIVAL OF BACTERIAL ISOLATES EXPOSED TO SIMULATED JOVIAN TRAPPED RADIATION BELT ELECTRONS AND SOLAR WIND PROTONS*

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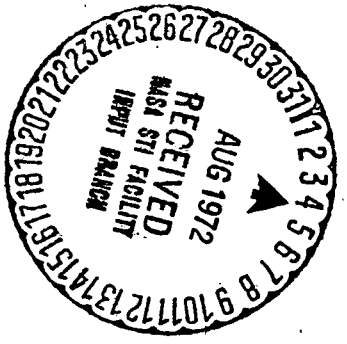

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Abstract

With missions to Jupiter, the spacecraft will be exposed for extended duration to solar wind radiation and the Jovian trapped radiation belt. This study is designed to determine the effect of these radiation environments on spacecraft bacterial isolates. The information can be used in the probability of contamination analysis for these missions.

A bacterial subpopulation from Mariner Mars 1971 spacecraft (nine sporeforming and three nonsporeforming isolates) plus two comparative organisms, Staphylococcus epidermidis ATCC 17917 and a strain of Bacillus subtilis var. niger, were exposed to 2-, 12-, and 25-MeV electrons at different doses with simultaneous exposure to a vacuum of 1.3×10^{-4} N m^{-2} at 20 and -20°C.

The radioresistance of the subpopulation was dependent on the isolate, dose, and energy of electrons. Temperature affected the radioresistance of only the sporeforming isolates.

Survival data indicated that spores were reduced approximately 1 log/1500 J kg^{-1}, while nonsporeforming isolates (micrococci) were reduced 1.5 to 2 logs/1500 J kg^{-1} with the exception of an apparent radioresistant isolate whose resistance approached that of the spores. The subpopulation was found to be less resistant to lower energy than to higher energy electrons.

The bacterial isolates were exposed to 3-keV protons under the same conditions as the electrons with a total fluence of 1.5×10^{13} p cm^{-2} and a dose rate of 8.6×10^{9} p cm^{-2} sec^{-1}. The results showed only 20% of S. epidermidis and 45% of B. subtilis populations survived exposure to the 3-keV protons, while the mean survival of the spacecraft subpopulation was 45% with a range from 31.8% (nonsporeformer) to 64.8% (nonsporeformer). No significant difference existed between sporeforming and nonsporeforming isolates.

1.0 Introduction

The development of mission planetary quarantine parameters consistent with a defined planetary quarantine constraint for any mission to a planet of biological interest follows three major steps: (1) identification of possible mission events which could result in contaminating the planet; (2) development of an allocation model, where the total allocation (planetary quarantine constraint) is suballocated to each contamination event; (3) estimation of the probability that the planet will be contaminated by the event. The probability of contamination is a function of two major factors. The first factor deals with the physical characteristics of the mission, such as the reliability of the spacecraft to perform the required mission maneuvers, the accuracy of the guidance and control, the extent of the meteoroid environment, etc. The second factor deals with the viable microbial burden associated with each contaminating event. The probability that viable organisms will be present at the time the contaminating event occurs is a function of the initial microbial load and its ability to survive the spacecraft environment.

This paper reports on part of the research being conducted to generate experimental data to determine the probability of spacecraft microbial isolates surviving space environments. These environments include launch pressure change, space vacuum and heat, solar electromagnetic radiation, and particle radiation. This paper will be limited to a discussion of particle radiation.

2.0 Technical Discussion

2.1 Background and Experimental Approach

With missions to Jupiter, the spacecraft will be exposed for an extended duration to solar wind radiation and the Jovian trapped radiation belt. In both these radiation environments the type, energy, and flux vary depending on the prediction model and the location within the environment. The Jovian trapped radiation belt is predicted by the model used in this study [1] to be of **torus** shape extending approximately out along the equatorial plane as much as 30 Jupiter radii and to contain both electrons and protons. The prediction model indicates that the energy and flux levels of electrons and protons increase as distance from the planet decreases. The current model predicts electron energies as high as 20 MeV with a flux level up to 10^7 e cm^{-2} sec^{-1} and protons with energies >400 MeV and flux levels $>10^8$ p cm^{-2} sec^{-1}.

The solar wind contains both electrons and protons with varying energies and flux levels, depending on the activity of the sun and the distance from the sun in the case of flux levels [2]. The energy of solar wind protons is predicted to be between 0.5 and 4 keV with fluxes between 2.6×10^8 and 1×10^{10} p cm^{-2} sec^{-1} at 1 AU for a quiet and maximum disturbed sun, respectively. The solar wind electrons have energies between 0.013 and 0.13 keV with flux levels of 1.7×10^9 and 2×10^{11} e cm^{-2} sec^{-1} at 1 AU for a quiet and maximum disturbed sun, respectively.

For any mission where the planetary quarantine analysis considers the effect of these radiation environments on the probability of survival of microorganisms, there must be an understanding of certain questions for

these probabilities to be meaningful or useful: (1) what is the relationship of different types of radiation, energy levels, dose rates, and temperature on survival of microorganisms; and (2) what is the relative radioresistance of microorganisms naturally occurring on spacecraft hardware.

In an attempt to answer some of these questions, spacecraft bacterial isolates were exposed to electrons similar to those in the Jovian trapped radiation belt and protons at a particular energy found in the solar wind.

2.2 Test Procedures

A bacterial subpopulation recovered from Mariner Mars 1971 spacecraft (nine sporeforming and three nonsporeforming isolates) plus two comparative organisms, Staphylococcus epidermidis ATCC 17917 and a strain of Bacillus subtilis var. niger, were exposed to 2-, 12-, and 25-MeV electrons at 1500, 3000, and 4500 J kg^{-1} (150, 300, and 450 krad*) with a flux level of 10^{10} e cm^{-2} sec^{-1}. The bacterial isolates were simultaneously exposed to a vacuum of 1.3×10^{-4} N m^{-2} (10^{-6} torr) at 20 and -20°C. The same isolates were exposed to 3-keV protons under the same conditions as the electrons, with a total fluence of 1.5×10^{13} p cm^{-2} and at a flux level of 8.6×10^{9} p cm^{-2} sec^{-1}.

The sporeforming isolates were sporulated in the liquid synthetic medium of Lazzarini and Santangelo [3] modified by the addition of l-methonine and l-tryptophan (25 mg cm^{-3}). The spores were washed

*krads based on the energy absorbed in tissue.

seven times with sterile distilled water and finally resuspended in 95% ethanol. Lawns of the nonsporeforming isolates were produced on Trypticase Soy Agar (TSA) with 48-hr incubation at 37°C, after which the cells were harvested and washed with distilled water four times with final resuspension in distilled water.

A planchet (sample holder) was inoculated with either 10^5 spores or 10^6 vegetative cells. The inoculum was allowed to air dry in an environmentally controlled room (45% relative humidity, 21°C). The planchets were placed into the test fixture and exposed to the selected test conditions. At the conclusion of a test, a planchet was assayed for viable microorganisms by placing the planchet into 10 cm^3 of 0.1% sterile peptone water and insonating at 25 kHz for 12 min. Serial 10-fold dilutions of this suspension were plated in triplicate by the pour plate method using TSA.

A vacuum chamber was constructed from stainless steel tubing with aluminum face plates. The test fixture, a planchet retainer collar made from aluminum, was mounted to the chamber flange. Thermoelectric coolers, placed between the test fixture and flange, controlled temperatures across the test fixture so that one half of the fixture was maintained at 20°C while the other half of the fixture was maintained at -20°C. During chamber operation, the pressure was in the 1.3×10^{-4} N m^{-2} range in 15 minutes. At the conclusion of a test, the chamber was backfilled to ambient pressure (10^5 N m^{-2}) with dry nitrogen.

The vacuum chamber was constructed so that two flanges with test fixtures were employed for a test: one flange exposed to vacuum, temperature, and radiation and the other flange exposed only to vacuum and temperature. The radiation effect was determined by comparing microbial survival occurring on the two flanges. All test data were subjected to an analysis of variance with the effects being significantly different at the 0.05 level of probability.

The 2-MeV electrons were produced by a dynamitron, a direct current accelerator. The flux profile was established and the exposure was monitored with the use of Faraday cups. The 12- and 25-MeV electrons were produced by a LINAC operated at 15 pulses sec^{-1} and a pulse width of 2 to 3 μsec. The flux profile was established prior to each run with LiF thermoluminescent detectors (TLD). The magnitude of the flux in these maps and during the test runs was standardized by a center-mounted PIN diode. The 3-keV protons were generated as an essentially direct current beam by a radio-frequency ion source. The flux in the beam spot was established by a Faraday cup and monitored by the source current. The spatial profile was determined by the properties of a scan system that was employed to cover the test plane.

3.0 Results

3.1 Electrons

The radiosensitivity of the bacterial subpopulation to electrons was found to be a function of the individual isolate, total exposure, and the energy of the electrons. The higher temperature was found to significantly increase the sensitivity of spore isolates but had no significant effect on nonsporeforming isolates.

The effect of 1500, 3000, and 4500 J kg^{-1} exposures across all three energies of electrons on spores of B. subtilis and the sporeforming spacecraft subpopulation is shown in Figure 1a. The mean survival fraction for the spacecraft isolates is plotted, with those isolates exhibiting maximum and minimum resistance identified at each dose. The survival curve for B. subtilis indicates an approximate one log reduction in the population for each 1500 J kg^{-1} exposure. The mean survival of the spacecraft isolate subpopulation indicates that these isolates were more resistant to electron irradiation than B. subtilis. The data showed that no individual isolate was the most or least resistant to all exposures.

The effect of the electrons on the nonsporeforming organisms is shown in Figure 1b. The survival curve for S. epidermidis indicates that populations of this organism were reduced approximately two logs for each 1500 J kg^{-1} dose. The nonsporeforming isolates exhibited a greater resistance than S. epidermidis to the electron irradiation, with the resistance of isolate No. 5 being similar to spores of B. subtilis.

The sensitivity to dose of the sporeforming and nonsporeforming isolates was a function of electron energy (Table 1). At all doses the spores were least resistant to 2-MeV electrons. The spores were also less resistant to 12 MeV than to 25 MeV at 3000 and 4500 J kg^{-1}. The percent reduction of the population was greatest at the lowest electron energy for each additional 1500 J kg^{-1} exposure.

The interaction between electron energy and exposure for the nonsporeforming isolates was different from that of the spore isolates (Table 1). It was found that electron energy did not affect the

radioresistance of the isolates at 1500 J kg^{-1} and that there was no significant difference between 2 and 25 MeV electrons at 3000 J kg^{-1}. The nonsporeforming isolates were more sensitive to 12 MeV electrons at 3000 and 4500 J kg^{-1}.

The effect of electron energy was dependent on the individual isolates. This dependence for the sporeforming isolates is shown in Figure 2. All spore isolates were significantly more sensitive to 2 MeV than to 12 or 25 MeV electrons. Exposure of the isolates to 12- and 25-MeV electron energies indicated that four of the 10 isolates (Numbers 8, 11, 13, and 18) were not significantly affected by higher energy, which suggests that for some organisms the effect of electron energy does not increase beyond a particular energy level.

The radioresistance to electrons of only the sporeforming isolates was significantly increased at -20°C compared with 20°C (Table 2). This temperature effect on spores was influenced by both the dose and the electron energy. Survival was significantly greater at -20°C than at 20°C with 3000 and 4500 J kg^{-1} doses (Table 2a). The data also indicated that with increasing electron energies temperature became less effective with no significant effect at 25 MeV (Table 2b).

3.2 3-keV Protons

As previously indicated, the spacecraft bacterial subpopulation and the two comparative organisms were irradiated with 3-keV protons at one exposure (1.5×10^{13} p cm^{-2}) and flux (8.6×10^{9} p cm^{-2} sec^{-1}). An analysis of these data indicated that all organisms were significantly affected and that sensitivity was dependent on isolate and temperature.

The percent survival of the spacecraft isolates and comparative organisms is shown in Figure 3. The percent survival ranged from 20.2% for S. epidermidis to 64.8% for spacecraft isolate No. 5, a nonsporeforming bacterium. The percent survival for B. subtilis (45%) was the same as the mean survival of the spacecraft subpopulation. In contrast to the effect of electrons, no significance in the radiosensitivity of sporeforming and nonsporeforming isolates to 3-keV protons was noted. Temperature was found to have a significant effect on radioresistance across all organisms, with approximately 10% more surviving irradiation at -20°C than at 20°C.

4.0 Summary

Studies were conducted to generate experimental data for the determination of the probability of survival of initial spacecraft bacterial burden when exposed to the Jovian trapped electron radiation belt and the solar wind proton radiation. These studies begin to answer questions related to the effects of different types of radiation, energy levels, dose rates, and temperature on the survival of microorganisms that may be present on the spacecraft.

Based upon results from these studies, the lowest electron energy (2 MeV) tested was the most effective in reducing initial population of spacecraft bacterial isolates. In general, electron dose was effective in reducing initial spore populations 1 log and vegetative cells 1.5 logs for each 1500 J kg^{-1} dose. Temperature, although not significant with vegetative cells, did affect survival of spores, with the spores surviving better at -20°C than at 20°C.

Protons at an energy similar to those present in the solar wind were effective in reducing initial populations of both sporeforming and nonsporeforming spacecraft isolates.

The results of these studies indicate that the probability of the spacecraft bacterial burden surviving the Jovian trapped electron belt will be a function of the location and time in the belt and the temperature of the spacecraft surfaces. The data indicates that the solar wind radiation can be considered as an additional parameter in reducing surface bacterial burden.

The comparative organisms, B. subtilis and S. epidermidis, were not found to be representive in radiosensitivity, compared with the spacecraft bacterial isolates. This emphasizes the requirement that with studies of this nature, indigenous microbial populations should be utilized.

5.0 Acknowledgements

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Table 1. Effect of electron energy and dose on spacecraft bacterial isolates exposed to vacuum

Dose, J kg^{-1}	Electron energy, MeV		
	2	12	25
Spores			
1500	10.80_{e}[1,2]	27.90_{g}	28.50_{g}
3000	0.70_{b}	5.35_{d}	13.90_{f}
4500	0.07_{a}	2.53_{c}	4.69_{d}
Nonsporeformers			
1500	2.40_{q}	4.74_{q}	7.52_{q}
3000	0.055_{p}	0.013_{o}	0.130_{p}
4500	0.011_{n}	0.000_{m}	0.070_{p}

[1] Mean percent survival.

[2] Mean followed by same letter not significantly different at 0.05 level of probability.

Table 2. Effect of electron energy, dose, and temperature on spores of spacecraft bacterial isolates exposed to vacuum

(a) Dose		
Dose, J kg^{-1}	Temperature, °C	
	+20	-20
1500	19.8_{e}[1,2]	21.4_{e}
3000	3.19_{c}	4.35_{d}
4500	0.687_{a}	3.01_{b}
(b) Electron energy		
Electron energy, MeV	Temperature, °C	
	+20	-20
2	0.564_{a}[1,2]	1.14_{b}
12	5.86_{c}	8.89_{d}
25	13.01_{e}	12.0_{e}

[1] Mean percent survival.

[2] Means followed by same letter not significantly different at 0.05 level of probability.

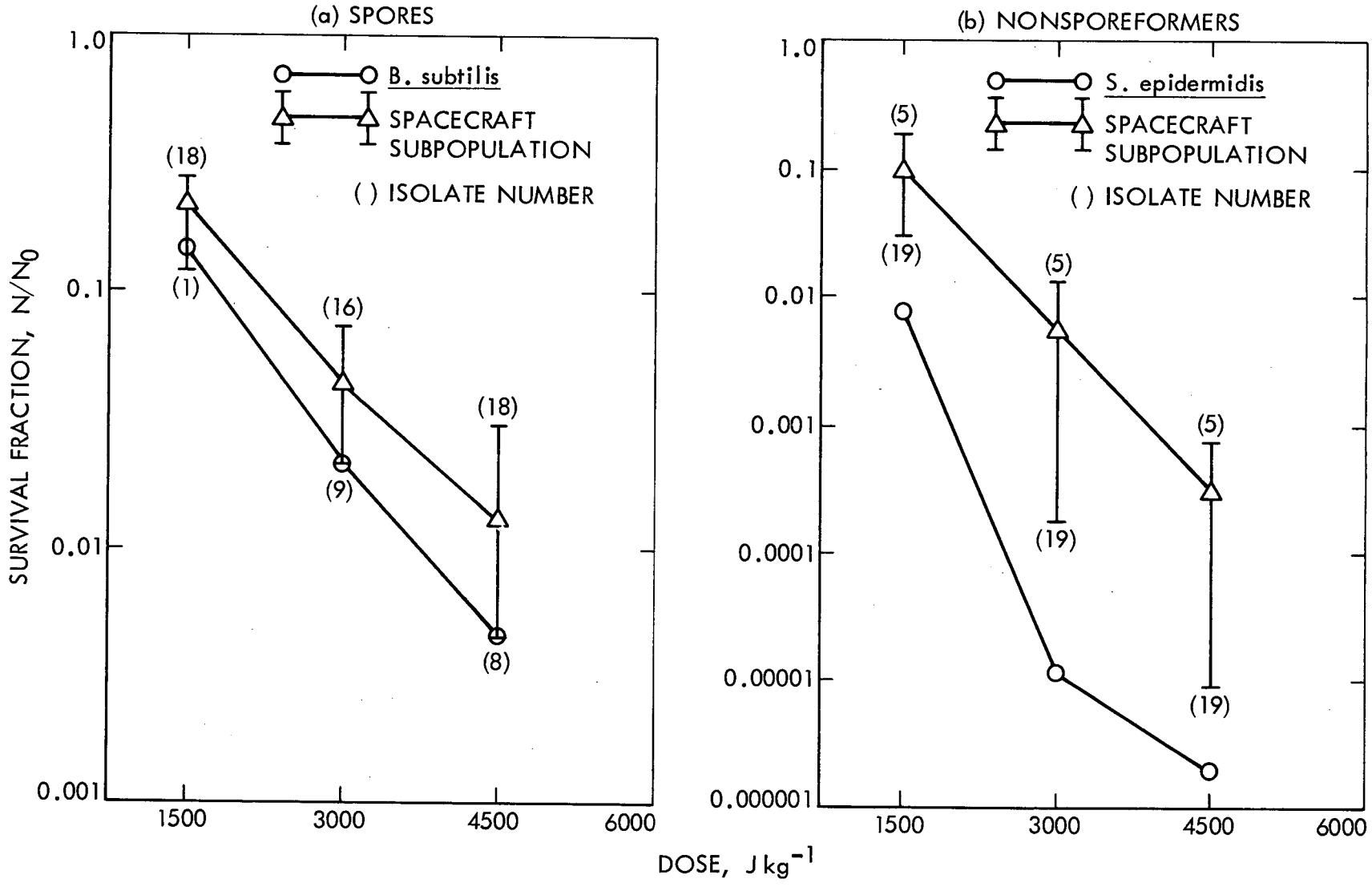

Figure 1. Effect of electron irradiation dose on spacecraft bacterial isolates exposed to vacuum

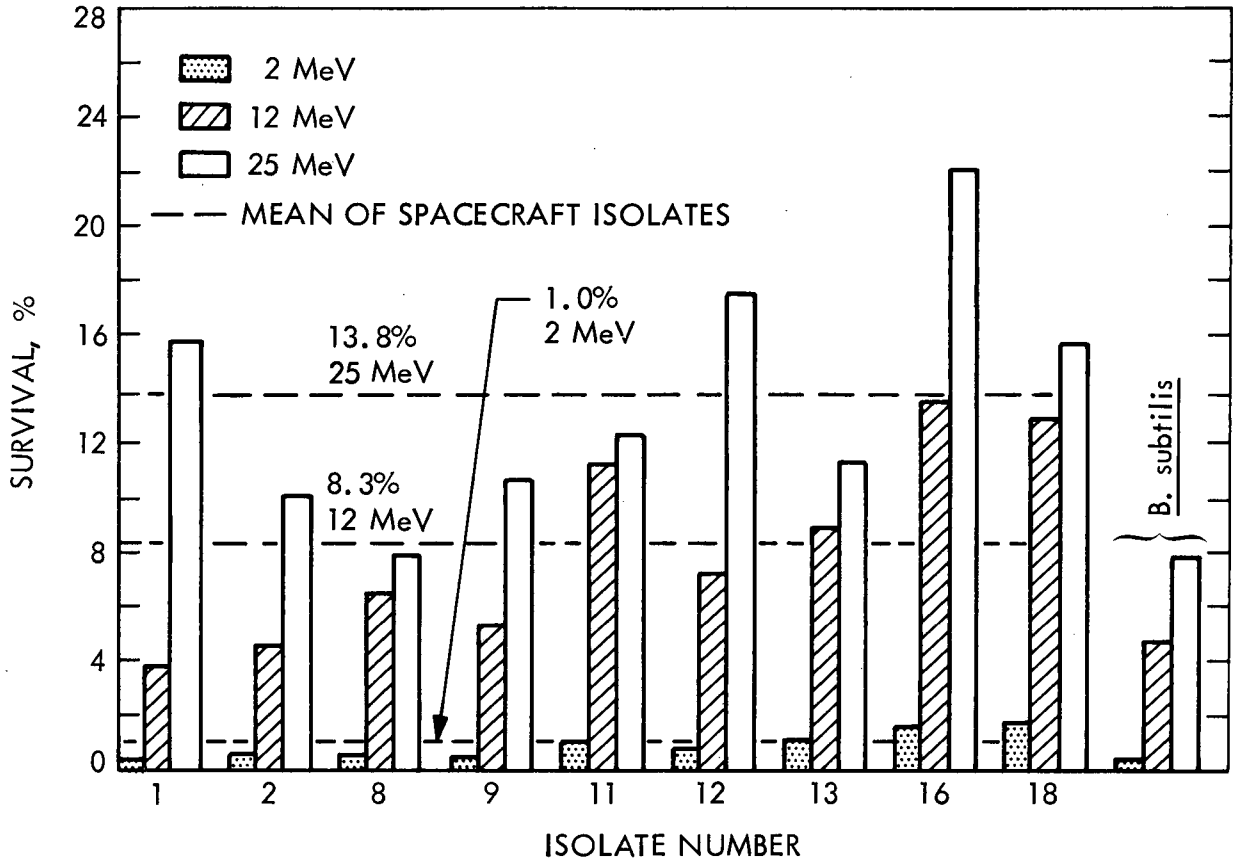

Figure 2. Effect of electron energy on spores of spacecraft bacterial isolates exposed to vacuum

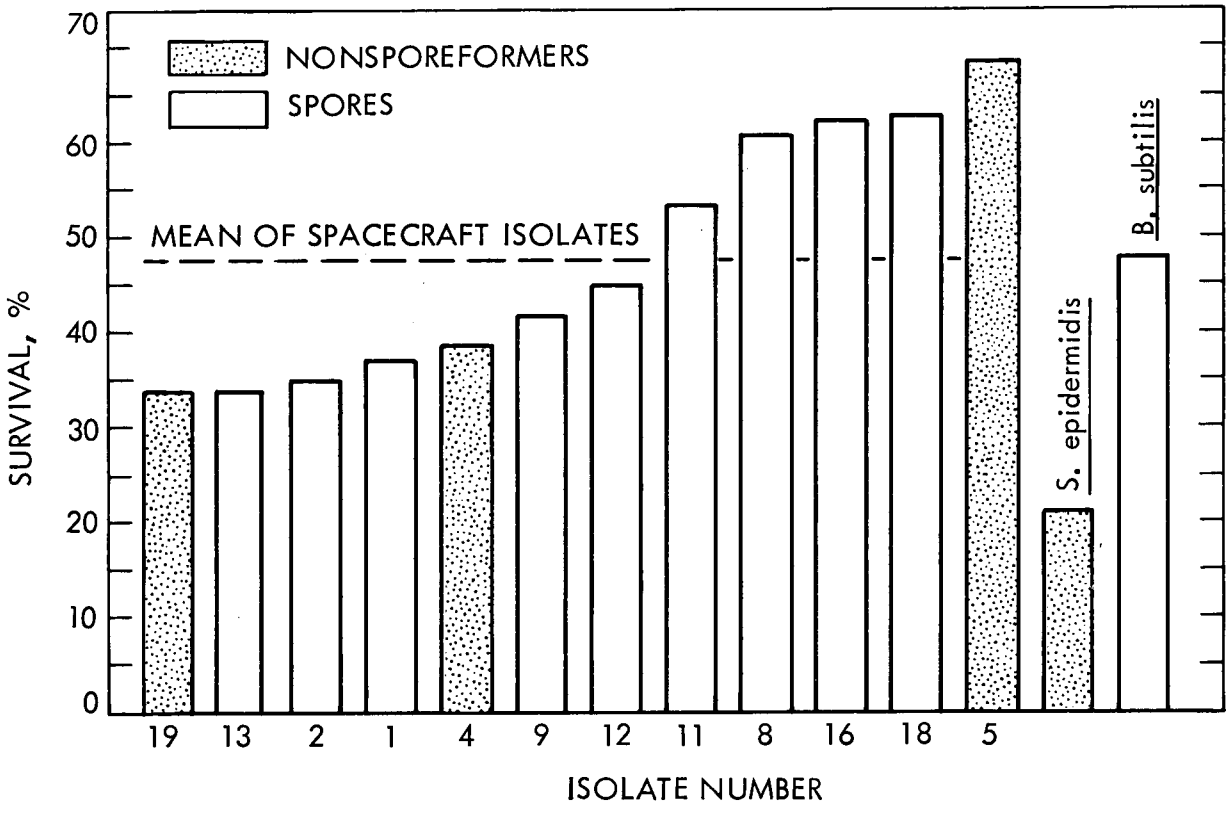

Figure 3. Effect of 3-keV protons on spacecraft bacterial isolates exposed to vacuum